Relationship Between Colour Improvement and Metallo-Chlorophyll Complexes During Blanching of Peas and Broccoli

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ABSTRACT

The influence of adding different levels of zinc chloride ranged between 100 and 500ppm and blanching time (1, 3 and 6 mins) at 85°C on the physical and structural aspects of chlorophyll pigments in peas and broccoli were investigated. It was found that when 300 and 100 ppm zinc chloride were added to the blanching water of peas and broccoli respectively to be satisfactory enough in retaining the green- colour of these vegetables.

Hunter colour "-a*" values were found to be adequately represent the colour changes. These values were used to describe the results in terms of an exponential model. Raman spectroscopy was also applied to confirm the presence of Zn-chlorophyll complexes which protect the chlorophylls from converting into other derivatives.

The residual zinc chloride (ppm) in the tissues of peas and broccoli after blanching was found to be 47.55 and 10.13 ppm, respectively. Therefore, double benefits regarding fortification with zinc and retaining green pigments were achieved.

***Keywords:** chlorophyll retention, pigment characteristics, blanching, zinc chloride, Raman spectroscopy, Hunter colour analysis, peas, broccoli.*

INTRODUCTION

During heat processing of vegetables, chlorophyll pigments are totally degraded to yellow olive coloured pheophytins and pyropheophytins (Shwartz & Von Elbe, 1983). Various efforts to preserve the green chlorophylls have been carried out. Factors such as pH, temperature, presence of salts, enzymes and surface active ions, influence the stability of chlorophylls. Chlorophyll has better stability at higher pH (Rayan- Stoneham & Tong, 2000). Salts of magnesium, calcium, sodium, ammonium and some surface active agents are known to have some stabilizing effect on chlorophyll degradation (Woolf, 1979). Some authors used manganese cations as a possible processing aid to improve the darkening process of ripe green olives (Romero *et al.*, 2001); whereas other studies emphasized that Zn++ or copper ions might be responsible for the green staining that occurred during the processing of table olives (Guerrero *et al.*, 2002).

In general, the stabilizing effect of 2% NaCl on green colour degradation has been kinetically studied, but the actual mechanisms could not be revealed (Nisha *et al.*, 2004). Zinc salts were deliberately added to blanching water to improve green colour in a commercial canning process for peas, beans and spinach (Segner *et al.*,1984). This process known as veri-green and produced canned vegetables with brighter colour as compared to conventionally canned vegetables This veri-green vegetables were attributed to the formation of zinc-pheophytin and zinc pyropheophytin a (Von Elbe *et al.*, 1986). Moreover, Laborde & Von Elbe 1996, proposed this technology to improve colour quality of green beans by blanching the vegetables with an aqueous solutions containing zinc ions and then packing them into a container filled with the same solution to keep the brightness of the green colour. Ngo & Zhao (2005) applied the same technology for producing a peel on green pears using zinc ions as a processing aid.

Zinc is an essential trace mineral element in the human body. It plays an important role in body growth and developments and helps to maintain a healthy immune system. The world health organization, food and agricultural organization and atomic energy association (WHO, FAO & IAEA,

2002) estimated that zinc deficiency affect about $^1/_3$ of the world's population particularly infants, pregnant and lactating women as well as elderly persons. The current dietary reference intake (DRI) value for zinc is 11 mg/day for adults; zinc has low toxicity, although acute symptoms and lethargy may be observed when large doses (i.e > 1g) are consumed (Brown & Wuehler, 2000).

Recently, with the aid of new instrumental techniques, it is now possible to characterized zn- chlorophyll complexes by either using Raman spectroscopy or by using fast ionization bombardment (FIB) combined with collision activation and tendom mass spectrometry (MS/MS). In the first technique, Raman scattering involves excitation of a molecule with a photon light (usually from a laser source) that is then scattered with a change in energy from the excitation photon by the vibrational transitions correspond to the different stretching, bending, wagging, deformation and other types of the molecules.

The second technique involves a tendom mass spectrometer which is a specialized instrument that detects molecules by measuring their weight (mass). The conventional configuration of this instrument contains two mass spectrometers separated by a chamber where the compounds are fragmented gently. The MS/MS tendom mass spectrometry is analog to the GC/MS, in that the separation of individual components of a mixture is achieved within a single instrument, but in this instance, a mass spectrometer substitutes for the chromatograph. The compounds must firstly be ionized in order to be separated. For this purpose a soft ionization technique is used. This was achieved by fast ion atom bombardment (Hunt *et al.*,1982).

Therefore, the present study was carried out to investigate the influence of adding different levels of zinc chloride to the blanching water as a food processing aid on the colour characteristics of peas and broccoli. These vegetables were selected as examples of chlorophyll rich vegetables. Elucidating the structure of these metallo-chlorophylls complexes were also investigated using FT- Raman spectroscopy technique.

MATERIALS AND METHODS

Both fresh green peas (*Pisum sativum* spp) and broccoli (*Brassica oleracea*, L) were obtained from the local market in Giza-Cairo-Egypt and washed thoroughly to remove any fines that might affect the colour characteristics. Anhydrous purified zinc chloride powder was obtained from nice chemicals PVT. LTO, India.

Samples of two hundred grams of each of peas and broccoli were blanched in distilled water with different concentrations of $ZnCl_2$ at 85°C. Samples were then rinsed with fresh water and cooled to room temperature. Two ways completely randomly factorial design with three replications were conducted to investigate, the effect of $ZnCl_2$ concentrations (100, 200, 300, 400 and 500 ppm) and blanching time (1,3 and 6 mins) on the colour retention. The zinc salt used in this study was zinc chloride self affirmed GRAS (generally recognized as safe). This salt could be used safely as a nutrient supplements in foods for fortification. Hence, zinc chloride used in the blanching processing of vegetables does not only retain green colour but also provides additional benefits.

Analytical methods: Chlorophyll pigments of fresh, water and zinc blanched samples of peas and broccoli were extracted according to the protocol outlined by Canjura *et al.* (1999). Changes that occurred in the structure of chlorophyll were confirmed and elucidated using FT- Raman spectroscopy, after integrating the peaks representing acetone. The FT – Raman spectra of the previously mentioned samples were obtained using a Nicolet 870 spectrometer with the Nicolet Raman module 32B (Madison, wl, USA) and ND- YAG laser source operated at 1064 nm with a maximum power at 0.7 w. The samples were used without further preparation in a NMR glass tube. The calibration was carried out in two possible means. 1st mean by internal calibration using He- Ne laser beam and 2nd mean by external calibration using polystyrene reference sample. Spectra were obtained in Raman shift range between 200 and 4000 cm^{-1}. The system was operated using Omnic 5.1 software and the experiments were replicated three times.

Changes that occurred in the colour values of the blanched peas and broccoli samples as a function of both zinc chloride concentration (ppm) and blanching time (mins) were followed up using a tristimulus colour analyzers (hunter lab scan XE, Reston VA). The instrument was calibrated using a standard white tile

(x= 77.26, y = 81.94 and z = 88.14). The colour was measured in terms of lightness L^*, redness a^* and yellowness b^*.

For measuring the zinc content in both fresh and blanched samples, peas and broccoli were pureed using a blender and dried in a mechanical conventional oven at 50°C for 2 days and ground into powders. Then, this assay was carried out using the atomic absorption – spectrophotometeric method as described by AOAC, (1995).

Kinetic calculations: Since the major colour in most of the vegetables under study is green; hunter –a* values were considered as the best visual parameter to describe the rate of green colour improvement. Therefore, the following equation described by Nisha *et al.* (2004), was applied to simulate the obtained results.

$$\text{Ln}\,(-a / -a_0) = kt \qquad \text{.............. (1)}$$

Where

a = hunter – a value at time t (dimensionless)

a_0 = hunter – a_0 value at time zero (dimensionless)

K = rate constant for retention of green colour. $(\text{min})^{-1}$

t = reaction time (min).

RESULTS AND DISCUSSION

Our preliminary studies revealed that, the most critical stage of using zinc for retaining green pigments in both green peas and broccoli was during the blanching process. By properly controlling Zn^{++} concentration in blanching solution and the period of blanching, the green pigments can be successfully retained to minimize the zinc content in the final product; it is advisable to be used as a processing aid during blanching (Ngo & Zhao, 2005).

Figures (1, 2 and 3) show the effect of adding different levels of zinc chloride on the colour attributes measured as L* lightness, a* redness and b* blueness during blanching of the vegetables under study. It seems that different blanching time (mins) have contributed to enhance the colour values of these food materials, ($P<0.05$). The slight reduction in the L* values in the case of broccoli samples during blanching in different elevated levels of zinc chloride may be attributed to the effect of removal of air in the fine porous structure between the epidermal cells, that led to a change in surface reflecting properties (Woolfe, 1979). As indicated in Fig (2), b* values of the broccoli samples (ppm) were affected by applying zinc chloride solutions (ppm) as compared to peas. It seems that the green component was major in peas; in contrast to broccoli, where other pigments might be probably presented; their yellowness were lowered with further progress in zinc chloride levels at all the suggested blanching times.

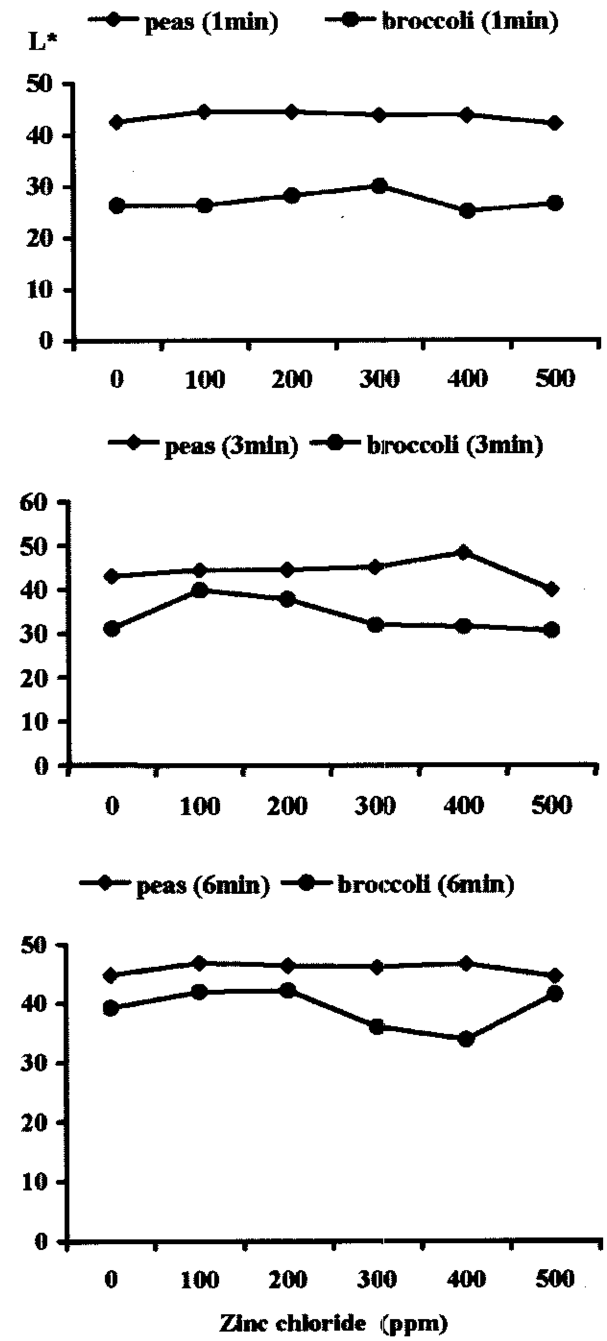


Fig. 1: Changes in the L* (lightness) values as a function of adding different levels of zinc chloride (ppm) and blanching time (mins)

Figure (3), illustrates the influence of zinc chloride treatment on the intense of the green colour of peas and broccoli samples. After blanching peas in water containing 300 (ppm) zinc chloride at 85C° for 6 mins, a maximum 143% enhancement in a* values was achieved.

Regarding broccoli samples; 100 (ppm) of zinc chloride under the aforementioned conditions

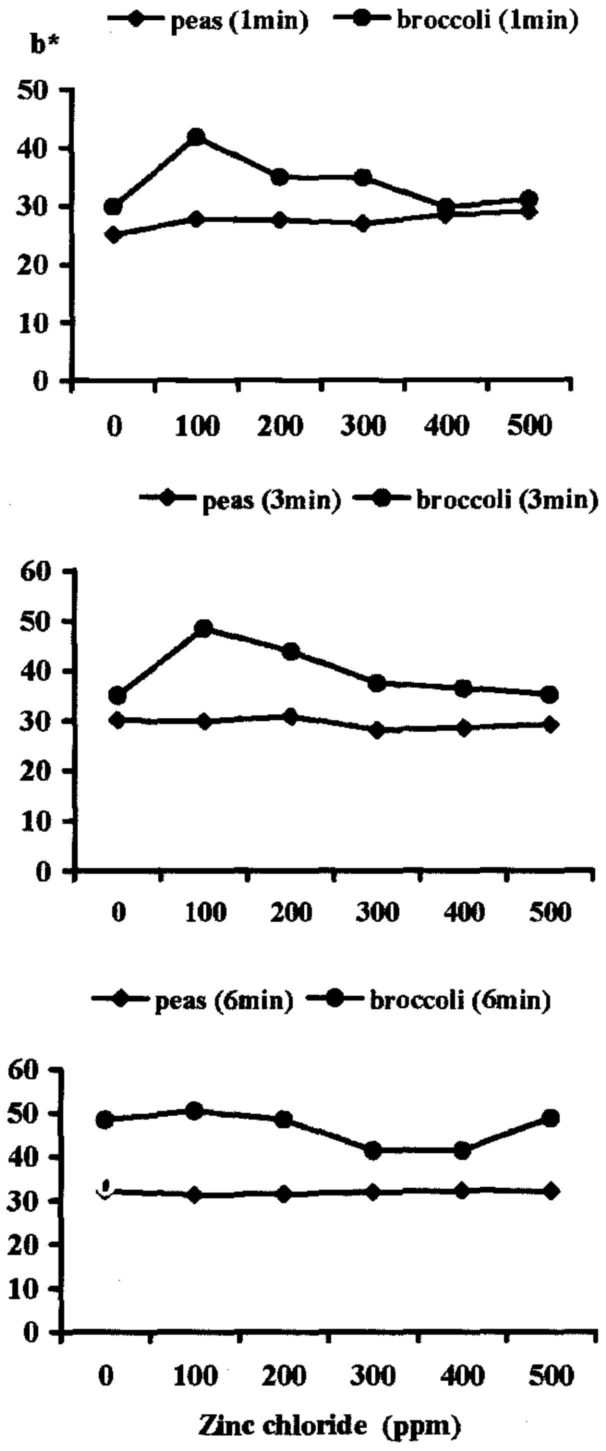


Fig. 2: Changes in the b* (yellowness) values as a function of adding different levels of zinc chloride (ppm) and blanching time (mins)

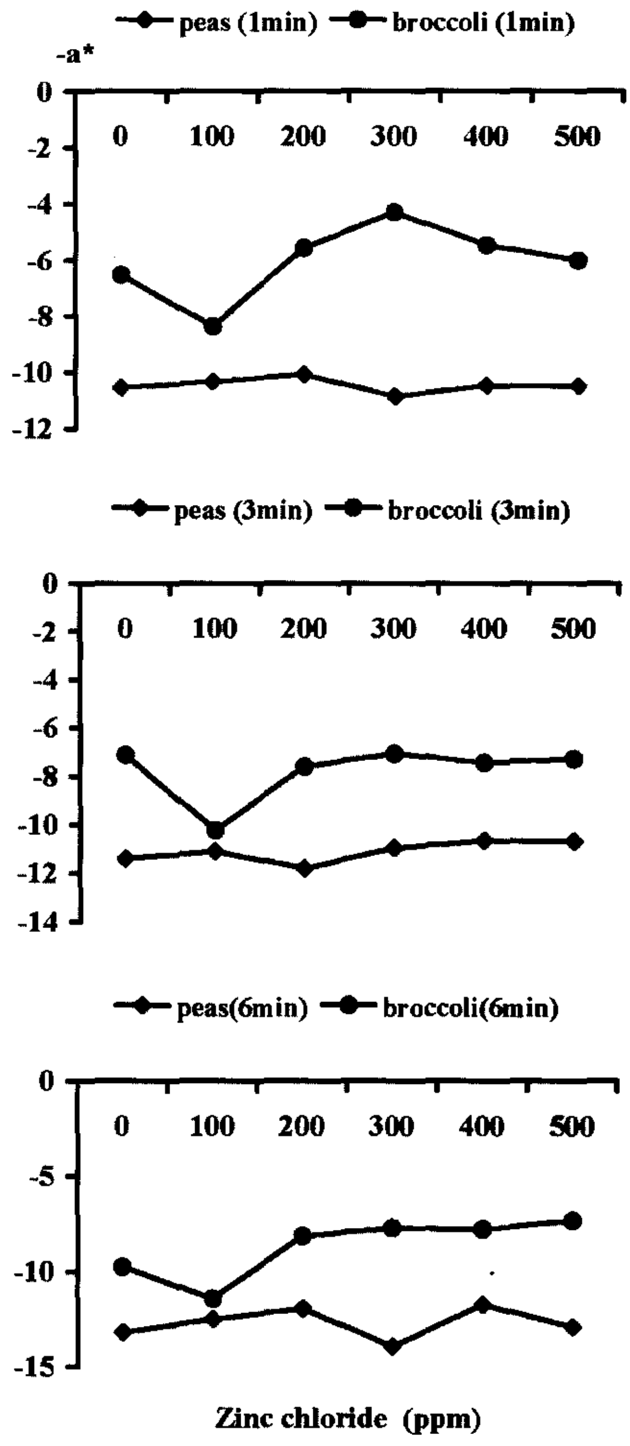


Fig. 3: Changes in the a* (Redness) values as a function of adding different levels of zinc chloride (ppm) and blanching time (mins)

were satisfactory enough for obtaining 195% (as compared to the initial values) enhancement in the green colour value a*. Since, the major colour of both broccoli and peas is green and the aim of this work was directed towards the use of zinc ions to preserve the green colour during blanching. The a* values were fitted to an exponential model to describe the retention of the green pigments. Table (1) shows the statistical results of this fitted model. The highest reaction rates were obtained, when using 300mg ml^{-1} and 100 mg. mL^{-1} zinc chlorides were added to the blanching solutions. The reaction

Table 1: Rate constants (k min^{-1}), correlation coefficients (R^2) probability (P) and standard error (SE) values for the retention of green colour (a*) fragments of both broccoli and peas

Zinc (ppm) concn.	BT (min)	Broccoli					Peas				
		a^*/a_0^*	K (min^{-1})	R^2	P	SE	a^*/a_0^*	K (min)$^{-1}$	R^2	P	SE
0	0	1					1				
	1	1.11	0.076	0.979	0.0051	0.035	1.09	0.049	0.986	0.003	0.019
	3	1.21					1.18				
	6	1.61					1.36				
100	0	1					1				
	1	1.43	0.098	0.799	0.052	0.160	1.07	0.038	0.988	0.004	0.016
	3	1.74					1.15				
	6	1.95					1.27				
200	0	1					1				
	1	1.10	0.046	0.828	0.045	0.067	1.04	0.037	0.833	0.043	0.055
	3	1.29					1.22				
	6	1.32					1.24				
300	0	1					1				
	1	1.10	0.043	0.943	0.014	0.034	1.13	0.054	0.9178	0.021	0.052
	3	1.20					1.14				
	6	1.31					1.43				
400	0	1					0				
	1	1.10	0.045	0.862	0.036	0.099	1.08	0.029	0.9342	0.016	0.025
	3	1.27					1.10				
	6	1.32					1.22				
500	0	1					1				
	1	1.02	0.042	0.818	0.048	0.065	1.09	0.034	0.9347	0.016	0.029
	3	1.24					1.11				
	6	1.26					1.26				

BT = Blanching time (min)

rates were (0.0540 min^{-1}) and (0.098 min^{-1}) for peas and broccoli, respectively. Results suggest that steric hindrance from peripheral groups and charge distribution on the tetrapyrrole ring had an effect on the reaction rate of chlorophyll derivatives with zinc ions (Tonucci & Von Elbe, 1992).

So, it should be emphasized that, thermal treatment is essential when using zinc for retaining green pigments, as heat is needed to dislodge Mg^{+2} and improve the diffusion of Zn^{+2} into the food material tissue, so that zinc complexes can be formed (Labored & Von Elbe, 1996). This observation suggested the use of Raman spectroscopy to elucidate, the structure of these green pigments before and after applying the $ZnCl_2$ solution to the blanching water.

Generally, in Raman spectra of chlorophylls, there are prominent bands in the regions (1620-1720 cm^{-1}) associated with the carbonyl stretching vibration; (1200-1400 cm^{-1}) related to stretching vibrations of the (C-C) and (C-N) groups; and in (1520-1620 cm-1) which resemble vibrations of the methane bridge (-CH). Vibrations of the MgN_4 core are observed below 700 cm^{-1}, mainly between 200 and 400 cm^{-1} (Twardowski & Anzenbacher, 1994).

In both the fresh and blanched peas chlorophyll extracts; the Raman revealed different spectra at; (1714.94, 1715.91) cm^{-1}, (1608.28,1603.21) cm^{-1}, (1446.46, 1444.89) cm^{-1} and (1361.73, 1222.64, 1197.8) cm^{-1}, respectively. On the other hand, different bands appeared in the ranges between (174.88-535.91) cm^{-1} in fresh and in (180.29-

472.16) cm^{-1} blanched samples. These variations could be related to organization of the MgN_4 core (Fig. 4, A and B).

In broccoli, the extracted chlorophylls showed the bands in both fresh and blanched samples at (1709.02, 1710.11), (1430.42, 1432.45) and (1227.43, 1223.24) cm^{-1}. The vibration of the MgN_4 core appeared in the ranges (397.17, 531.64) cm^{-1} and in (388.08- 531.88) cm^{-1}, respectively; (Fig. 4, D and E).

When zinc chloride was added to the water during blanching at 85°C, strong vibrations in the chlorophyll bands (1200-1720) cm^{-1} were obviously observed (Fig. 4, C and F).

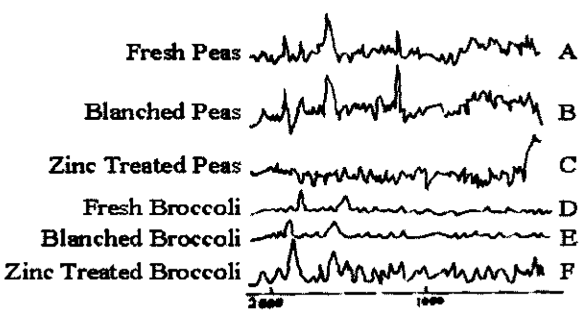
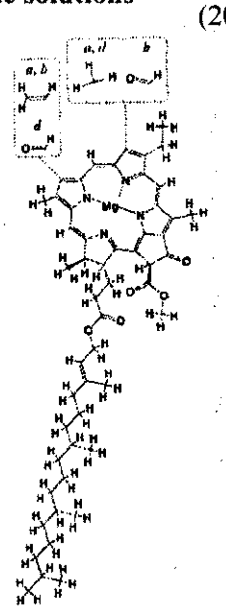


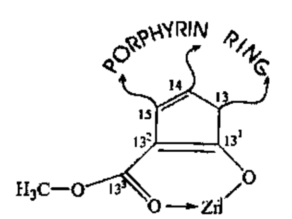
Fig. 4: Changes in the Raman spectra of both broccoli and peas chlorophylls as influenced by blanching in water and zinc chloride solutions

These vibrations indicated large changes in the electron density of the chlorophyll rings , which led to a conclusion that (C9) keto group is probably presented in an enol form. In such a complex the metal ions (Zn^{++}) bonds O- atom at the right and coordinate to another one at the left side. This coordination makes the double (carbonyl bond) longer and causes a shift towards these frequencies found in both of the two mentioned spectra of broccoli and peas (Petrovic *et al.*, 2006). Perhaps, there may be other proposed mechanism suggested by Kokube & Takahashi (2003). In that mechanism, the porphyrin units, which contain zinc and imadazolyl substituents are arranged in that ring, in the same manner as the magnesium containing chlorophylls unit are resembled in most of the vegetable chlorophyll pigments. There was also a charge separation center, where two parallel chlorophyll molecules faced each other with a slipped center to center deposition.

Table (2) shows, the zinc content in both peas and broccoli. Depending on zinc levels used in the blanching solution, the final zinc content (residual) was found to be 47.547 ppm in peas and 10.13 ppm in broccoli under the different processing conditions that are stated in the same Table. These aforementioned residual zinc content doses not contribute any toxicological impacts as reported by ZNCG (2004) since they recommended a total zinc intake



(A)
Common structure Of chlorophyll



(B)

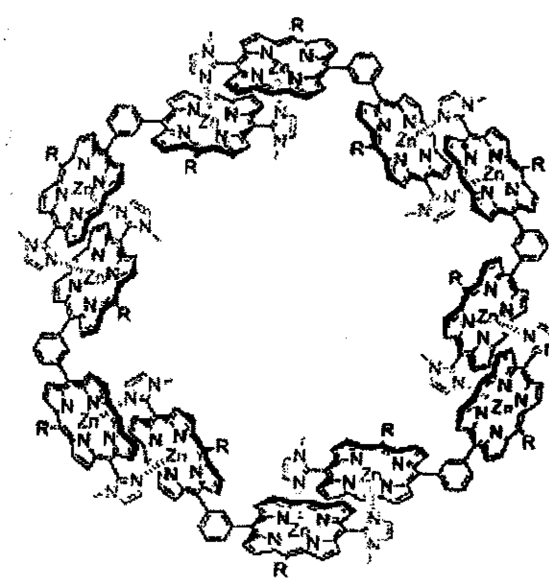


Fig. 5: Two proposed mechanisms (B, C) for the diffusion of the Zn^{++} ions into the chlorophyll to form Zn-chlorophyll complexes. These mechanisms are suggested by (Petrovic *et al.*, 2006) and (Kokube & Takaheshi 2003), respectively

of no more than 40 mg per day by adults. In both vegetables; a bright green colour was retained after blanching. It should be noted that not all the zinc was absorbed by tissues of both vegetables. Zinc may react with other constituents and only a fraction may react with chlorophyll to form Zn- chlorophyll complexes (Laborde & Van Elbe, 1990).

Table 2: Zinc content in the blanched peas and broccoli

Processing conditions	Zinc contents (ppm)
Peas*	
Control	14.202±1.6329
Blanching in 300 ppm $ZnCl_2$	47.547±2.449
Broccoli*	
Control	1.505±0.500
Blanching in 100 ppm $ZnCl_2$	10.13±4.082

*Both peas and broccoli were blanched at 85°C for 6 mins
Results are presented as means ± SE

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تحسين اللون وعلاقته بمعقد الكلورفيل المعدني أثناء سلق البسلة والبروكلى

منال فتحى سلامة وهشام أحمد محرم

قسم الصناعات الغذائية - المركز القومي للبحوث - الدقى - القاهرة

تم فى هذا البحث دراسة تأثير اضافة مستويات مختلفة من كلوريد الزنك (١٠٠-٥٠٠ جزء فى المليون) وزمن السلق (١، ٣، ٦ دقائق) عند ٨٥°م على الصفات الطبيعية والخصائص البنائية لصبغات الكلوروفيل الموجودة فى البسلة والبروكلي. وقد وجد أن احسن نسبة اضافة لكلوريد الزنك كانت نسبة ٣٠٠، ١٠٠ جزء فى المليون عند ٦ دقائق اثناء السلق لكل من البسلة والبروكلي على الترتيب وكانت هذه النسبة كافية للحفاظ على اللون اللأخضر لهذه الخضروات. ويمكن وصف درجة التحسن التى تحدث فى اللون الأخضر عند قياسه بجهاز هنتر بواسطة القيمة *a - والتى تعطي أحسن تمثيل للون بواسطة معادلة أسية. وقد استخدم جهاز الرامان للكشف عن وجود معقد الكلورفيل المرتبط بالزنك والذي يعمل على حماية الكلورفيل من التحول إلى مشتقات أخرى أثناء عملية السلق.

وكانت قيم كلوريد الزنك المتبقية فى البسلة والبروكلي فى حدود ٤٧,٥٥ : ١٠,٣٣ جزء فى المليون على الترتيب. أوضحت هذه الدراسة إمكانية تحسين اللون الاخضر فى الخضروات باستخدام مستويات مختلفة من الزنك وفى نفس الوقت الحفاظ على جودة اللون الاخضر فى أثناء السلق.